# PROMOTER

This application is the National Stage of International Application No. PCT/EP2008/005135 filed Jun. 25, 2008, which claims the benefit of EP 07012772.5 filed Jun. 29, 2007, which is hereby incorporated by reference in its entirety.

The current invention is in the field of protein expression and cell selection. It is herein reported a promoter with low promoter strength and thus with a limited expression of an operably linked coding nucleic acid.

## BACKGROUND OF THE INVENTION

The expression of proteins is a fundamental process in living cells. All information required for protein expression is provided by a single nucleic acid. This nucleic acid not only contains the information of the protein's amino acid sequence, it also provides the regulatory information required (e.g. the ribosomal binding site, the start and end-signals for transcription, splice signals, enhancer elements, etc.) including a promoter/promoter sequence.

A promoter is a nucleic acid that regulates the amount of transcription of a nucleic acid, e.g. encoding a polypeptide, to which it is operably linked, into pre-mRNA. It is a transcription control element, which is located around the RNA polymerase initiation site at the 5'-end of an operably linked coding sequence. From analysis of the SV40 early promoter it is known that recognition/binding sites for transcription activators are contained in promoters in segments consisting of 7-20 basepairs. One segment is the start site for RNA synthesis, e.g. the well known TATA-box. Other segments, located approximately 30-110 basepairs 5', i.e. upstream, to the start site for RNA synthesis, are defining the frequency of transcription initiation. A promoter at least requires one segment that initiates RNA synthesis at a specific site and in a defined direction, i.e. in 5' to 3' direction.

Known promoters are the lac-lpp, the ara-, the lac-, the tac-, the trc-, the trp-, the phoA-, the $P_{BAD}$-, the $\lambda_{PL}$-, the lpp-, and the T7-promoter. The SV40 promoter is a nucleic acid sequence derived from the genome of Simian (vacuolating) Virus 40. For the recombinant production of a heterologous polypeptide in a eukaryotic or prokaryotic cell normally one or more expression plasmids are introduced into the cell. The expression plasmid(s) comprises an expression cassette for the expression of a heterologous polypeptide and also an expression cassette for the expression of a selectable marker, which is required for the selection of transfected cells expressing the heterologous polypeptide. The synthesis of the heterologous polypeptide and of the selectable marker both requires a fraction of the cell's expression machinery's capacity.

As it is the aim to produce predominantly the heterologous polypeptide most of the available capacity of the cell's expression machinery should be allocated to the expression of the nucleic acid encoding the heterologous polypeptide. Only a minor amount should be used for the expression of the selectable marker. This allocation of expression capacity is done via the strength of the corresponding promoters. The stronger a promoter is the more of the operably linked nucleic acid is transcribed and thus translated. Therefore, it exists a need for promoters with adjustable or reducible promoter strength.

Taylor, W. E., et al. (Endocrinol. 137 (1996) 5407-5414) report human stem cell factor promoter deletion variants. In US patent application US 2007/0092968 novel hTMC promoter and vectors for the tumor-selective and high-efficient expression of cancer therapeutic genes is reported. Fromm et al. (J. Mol. Appl. Gen. 1 (1982) 457-481 and ibid 2 (1983) 127-135) report deletion mapping and deletion mutants of SV-40 early region promoter. Chitinase chitin-binding fragments are reported in U.S. Pat. No. 6,399,571. WO 99/62927 reports connective tissue growth factor-4.



## SUMMARY OF THE INVENTION

The first aspect of the current invention is a promoter having, i.e. with, a nucleic acid sequence of SEQ ID NO: 02 or SEQ ID NO: 03 or SEQ ID NO: 04 or SEQ ID NO: 06. In one embodiment the promoter has the nucleic acid sequence of SEQ ID NO: 04.

A second aspect of the current invention is a nucleic acid that has the nucleotide sequence of SEQ ID NO: 04 and that has a promoter strength of 20% or less compared to the wild-type SV40 promoter of SEQ ID NO: 05 when operably linked to the nucleic acid sequence of SEQ ID NO: 07 encoding the green-fluorescent-protein (GFP).

A further aspect of the current invention is a method for the selection of a cell comprising the following steps in this order:

a) transfecting a eukaryotic cell with a nucleic acid comprising
   i) a first expression cassette comprising a nucleic acid encoding a heterologous polypeptide,
   ii) a second expression cassette comprising a first nucleic acid of SEQ ID NO: 04 and a second nucleic acid encoding a selectable marker, whereby the first nucleic acid is operably linked to the second nucleic acid,
b) cultivating said transfected cell under conditions suitable for growth of the non-transfected eukaryotic cell,
c) selecting a cell propagating in step b) and also
   i) propagating under selective culture conditions, or
   ii) expressing the selectable marker.

In one embodiment of this aspect of the invention the eukaryotic cell is a mammalian cell. In a preferred embodiment the mammalian cell is a CHO cell, BHK cell, or PER.C6® cell, or HEK cell, or Sp2/0 cell. In another embodiment the heterologous polypeptide is an immunoglobulin, or an immunoglobulin-fragment, or an immunoglobulin-conjugate. In one embodiment the selectable marker is a neomycin-aminoglycoside phosphotransferase, or a hygromycin-phosphotransferase, or dLNGFR, or GFP.

A forth aspect of the current invention is a method for the expression of a heterologous polypeptide which comprises the following steps in this order:

a) transfecting a mammalian cell with a nucleic acid comprising an expression cassette comprising a first nucleic acid of SEQ ID NO: 02 or SEQ ID NO: 03 or SEQ ID NO: 04 or SEQ ID NO: 06 operably linked to a second nucleic acid encoding a heterologous polypeptide,
b) selecting a cell transfected in step a),
c) cultivating the selected cell under conditions suitable for the expression of the heterologous polypeptide,
d) recovering the heterologous polypeptide from the cell or the cultivation medium.

In one embodiment of this aspect of the current invention the mammalian cell is a CHO cell, a BHK cell, or a PER.C6® cell, or HEK cell, or Sp2/0 cell. In another embodiment the first nucleic acid is of SEQ ID NO: 04. In a further embodiment the second nucleic acid is encoding an immunoglobulin, or an immunoglobulin-fragment, or an immunoglobulin-conjugate. In still another embodiment the nucleic acid comprises a second expression cassette encoding a selectable marker.